Maastricht University

# Studies on blood coagulation factor V

Citation for published version (APA):

Kahn, M. J. P., & Hemker, H. C. (1970). Studies on blood coagulation factor V: IV. A partially purified factor V preparation from human plasma. *Coagulation*, *3*(1), 63-66.

**Document status and date:**
Published: 01/01/1970

**Document Version:**
Publisher's PDF, also known as Version of record

**Please check the document version of this publication:**

• A submitted manuscript is the version of the article upon submission and before peer-review. There can be important differences between the submitted version and the official published version of record. People interested in the research are advised to contact the author for the final version of the publication, or visit the DOI to the publisher's website.
• The final author version and the galley proof are versions of the publication after peer review.
• The final published version features the final layout of the paper including the volume, issue and page numbers.

Link to publication

**General rights**
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

• Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
• You may not further distribute the material or use it for any profit-making activity or commercial gain
• You may freely distribute the URL identifying the publication in the public portal.

If the publication is distributed under the terms of Article 25fa of the Dutch Copyright Act, indicated by the “Taverne” license above, please follow below link for the End User Agreement:

www.umlib.nl/taverne-license

**Take down policy**
If you believe that this document breaches copyright please contact us at:

repository@maastrichtuniversity.nl

providing details and we will investigate your claim.

Download date: 04 May. 2023

# Studies on blood coagulation factor V

## IV — A partially purified factor V preparation from human plasma

M.J.P. KAHN * and H.C. HEMKER

Laboratory for coagulation Biochemistry and Cardiovascular Biochemica Research. Clinic for Internal Medicine. University Hospital, LEIDEN.

Whereas factor V from bovine plasma can be readily purified (Ware, 1947 ; Aoki, 1963 ; Papahadjopoulos, 1964 ; Barton, 1967 ; Esnouf, 1967), the purification of human factor V is fraught with difficulties (Quick, 1943 ; Hjort, 1957 ; Surgenor and al., 1961 ; Blombäck, 1963 ; Weiss, 1965), principally because of the extreme lability of the purified factor. Yet a purified preparation of human factor V is indispensable for the investigation of the reaction mechanism of blood coagulation in man. This article describes the preparation of a protein fraction obtained from human plasma that is about 30 times purer than normal plasma on a protein basis and is minimally contaminated by other coagulation factors. This preparation was found to be suitable for use in further purification experiments as well as in studies on the interaction of factor V with other coagulation factors (Kahn and Hemker, 1970).

The method is based essentially on adsorption on ion-exchange cellulose and elution under conditions known to specificially preserve factor V activity, such as relatively high concentrations of $Ca^{++}$ or $Mg^{++}$ ions and the presence of glycerol (Esnouf and Jobin, 1967 ; Weiss, 1967).

## MATERIALS AND METHODS

### Preparation of ion-exchange celhiloses

The cellulose was suspended in twice-distilled water and washed three times on a Buchner funnel with 0.5 M HCl and 0.5 M NaOH alternately, after which it was resuspended in twice-distilled water and again thoroughly washed on a Buchner funnel until the pH of the wash water was 7 $\pm$ 0,1. The cellulose was then suspended in the buffer to be used for elution of the adsorbed protein later in the experiment (1 g cellulose on 50 ml buffer).

The slurry was stirred at room temperature for 5 to 12 hours, brought onto a Buchner funnel, and washed with the same buffer diluted 1 : 100 in water, using 20 times the volume of the original slurry.

The cake of cellulose was drained by suction on the funnel and the wet powder was used for adsorption. A sample of the wet powder was weighed, dried, and reweighed to estimate the weight proportions between wet and dry powder. The values given in the text refer to the dry weight of the cellulose.

The following ion-exchange celluloses were used :

— Triethylaminoethylester cellulose (TEAEC) ;

— Diethylaminoethylester cellulose (DEAEC) ;

— Carboxymethyl cellulose (CMC) ;

— Ecteola cellulose (ECTC).

### Adsorption and elution of the plasmas

A given amount of ion-exchange cellulose was mixed with the starting material after which the mixture was agitated for 20 min. at room temperature and then centrifuged for 10 min. (5,000 g) at 4 °C. The sediment was washed once with one starting volume of the eluting-buffer diluted 1 in 100, and then centrifuged in the same way.

The eluting-buffer was added to the sediment in a volume of 1/5$^{th}$ of the original material. Dialysis was

* Present address : Laboratory for Pharmacodynamics and Therapeutics. Free University. BRUSSELS (Belgium).

Article reçu le 24 septembre 1969 ; accepté le 10 décembre 1969.

**Les tirés à part peuvent être demandés au :** Dr H.C. HEMKER, Academisch Ziekenhuis Algemene Interne Geneeskunde, LEIDEN (PAYS-BAS).

**Table I**

A comparison of bovine and human material.

| | bovine | | human | |
|---|---|---|---|---|
| | units | % | units | % |
| Starting material ..... | 250 | 100 | 139 | 100 |
| Supernatant portion after adsorption with TEAEC .... | 107 | 43 | 9 | 6,5 |
| Eluate ..... | 47 | 19 | 2 | 1,5 |
| Supernatant portion after adsorption with CMC ..... | 91 | 36 | 11 | 8 |
| Eluate ..... | 28 | 11 | 1 | 0,7 |

Starting material : 200 ml fresh citrated plasma twice diluted with distilled water. Adsorption with 6 g cellulose.
Elution with 0.4 M phosphate buffer (pH 7), dialysis against distilled water.
Means of 5 experiments.

**Table II**

The effect of glycerol on the yield in factor V purification procedure.

| | with glycerol | without glycerol |
|---|---|---|
| Starting plasma (bovine, citrated) ..... | 100 % | 100 % |
| Supernatant portion after adsorption with Al $(OH)_3$ .. | 53 % | 40 % |
| Supernatant portion after adsorption with TEAEC .... | 10 % | 5 % |
| TEAEC eluate ..... | 21 % | 12 % |

Procedure : glycerol (when present) 50 % (v/v) Al $(OH)_3$ : 3 % (w/v) TEAEC : 2 % (w/v). Elution with 0.5 M $MgSO_4$ in 0.05 M tris — $H_2SO_4$ (pH 7).
Dialysis against 0.01 M $MgSO_4$ in 0.05 Tris — $H_2SO_4$ (pH 7).

**Table III**

Stability of a human factor V preparation during dialysis.

| Stabilizer | Dialysed against | Dialysis time (hours) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ½ | 1 | 2 | 3 | 6 | 10 | 24 | 36 | 60 | 90 | 150 |
| Glycerol 50 % $MgSO_4$ 0,5 M | Glycerol 50 % $MgSO_4$ 0,01 M | 80 | — | 78 | — | 76 | 54 | — | 39 | — | 48 | 29 |
| Glycerol 50 % $MgSO_4$ 0,5 M | Glycerol 50 % | 64 | 42 | 32 | — | — | — | 27 | 16 | 8 | 4 | < 1 |
| Glycerol 50 % $MgSO_4$ 0,5 M | $MgSO_4$ 0,01 M | 55 | 41 | — | — | — | 98 | 8 | < 1 | < 1 | — | — |

The pH was constant at 7.5 ± 0.1. Incubation temps was 25 °C.
Means of 2 experiments.

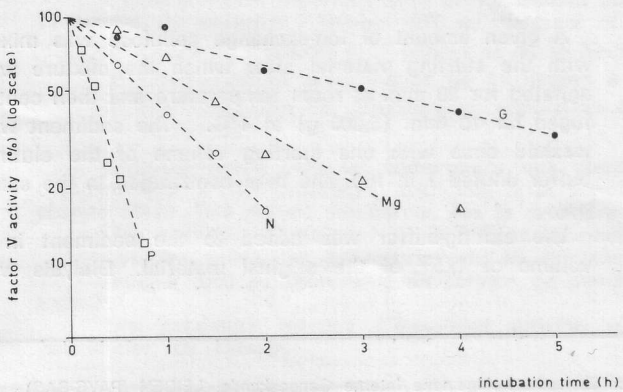


**Fig. 1.** — Disappearance of factor V under different conditions. The preparation was dialysed for 12 hours against Tris — $H_2SO_4$ buffer 0.05 M pH 7.5 with or without glycerol and/or $MgSO_4$ so as to obtain the desired final concentrations. Then the activity was arbitrarily said to be 100 %, and incubation at 37 °C was started. N : normal plasma ; P : preparation in 0.05 Tris $H_2SO_4$ buffer ; Mg : preparation in 0.5 M $MgSO_4$ ; G : preparation in 50 % (v/v) Glycerol.

carried out against 20 times the sample volume of twice-distilled water or buffer, as indicated in the next section, for 2 periods of 8 to 12 hours at 4 °C. Determination of coagulation factors and other procedures were carried out as indicated by Kahn and Hemker (1970).

One **unit** of factor V is defined as the amount present in 1 ml of fresh normal plasma having 100 % factor V activity (i.e. standard normal plasma) corrected for dilution with citrate.

## Experimental

Table I illustrates the fact that a procedure for preparation of factor V which can give satisfactory results with bovine plasma yields hardly any factor V activity with human material. Human factor V seems to be readily adsorbed, but is not found in the eluate. Variation of the elution buffer (pH of 6 ; 6.5 ; 7 ; 7.5 ; 8 ; 8.5, in molarities of 0.2 ; 0.3 ; 0.4 ; 0.5 ; addition of up to 2 M NaCl ; substitution of citrate buffers for phosphate buffers) failed to result in the appearance of any activity in the eluate with the use of human material. The factor V activity from human plasma probably is destroyed during the procedure.

All of the ion-exchange celluloses tested (TEAEC, DEAEC, CMC, ECTC) caused the disappearance of factor V from the starting material ; from none of them could activity be eluted in a satisfactory yield.

Because it is known that minute amounts of thrombin can adversely influence the activity of factor V (Surgenor and al., 1961), factors II, VII, IX and X were removed from the starting material by adsorption on 3 % Al $(OH)_3$ (w/v). This treatment removed about 50 % of the factor V in the starting material, but did not improve the finai yield of the procedure. The only condition under which an acceptable amount of activity was found in the eluate was elution with 0.5 M $MgSO_4$ in a 0.05 M Tris — $H_2SO_4$ buffer (pH 7.0) in the presence of 25 to 50 % glycerol.

The activity was more stable with than without glycerol, and the yield of the preparation was improved considerably. Because the Al $(OH)_3$ adsorption substantially reduced the content of contaminating coagulation factors, this step was included in the final preparation procedure chosen. The stabiliring properties of $MgSO_4$ and glycerol are illustrated in fig. 1. The necessity of dialysing against a fluid containing glycerol and $Mg^{++}$ ions is shown in table III.

## Description of the method

In its final form the method consisted of :

1. Dilution of fresh citrated plasma with an equal volume of glycerol.
2. Adsorption with 3 % Al $(OH)_3$ (w/v) followed by centrifugation for 10 min. at 12 000 g and 4 °C.
3. Dilution of the supernatant portion with an equal volume of twice-distilled water.
4. Adsorption with 2 % TEAE cellulose (w/v) prepared as described under methods, followed by centrifugation for 10 min. at 12.000 g and 4 °C.
5. Elution of the factor V from the sediment with 0.5 M $MgSO_4$ in 0.05 M tris — $H_2SO_4$ buffer, pH 7.0 containing 50 % (v/v) glycerol.
6. Dialysis against 0.01 M $MgSO_4$ in 0.05 M tris — $H_2SO_4$ buffer, pH 7, containing 50 % (v/v) glycerol, at 4 °C.

When concentrated 14 times, 14 preparations made with this procedure had the following mean composition : factor V : 40 % (varying between 20 and 64 %) ; factors VII-X : < 0,1 % ; factor II : < 0,1 % ; factor VIII : < 1,0 % ; factor IX : < 0,1 % ; factor XI : 4 % ; factor XII : 2 %. The mean purification on a protein basis was 30 times (between 18 and 46).

## DISCUSSION

The lability of human factor V makes it a most awkward protein to purify, but purification is an essential prerequisite for studies on the role of this factor in the coagulation process. Like bovine factor V, human factor V is stabilized by $Mg^{++}$ ions and glycerol.

This property makes it possible to obtain a preparation which is about 30 times purified on a protein basis and which contains as principal contaminating coagulation factors low concentrations of factors XI and XII only. The yield of the procedure is only about 15 % this is mainly due to adsorption of factor V onto Al $(OH)_3$ early in the procedure. We nevertheless did not eliminate this step because it prevented contamination of the final product by factors II, VII, IX and X.

A score of variations of the procedure were tested, but the one chosen here appeared to be the best.

## REFERENCES

[1] AOKI N., HARMISON C.R., SEEGERS W.H. — Properties of bovine Ac-globulin concentrates and methods of preparation. **Canad. J. Biochem. Physiol.**, 1963, **41**, 2409.

[2] BARTON P.G. — The preparation and the properties of a stable factor V from bovine plasma. **Biochim. Biophys. Acta**, 1967, **133**, 506.

[3] BERGSAGEL D.E., NOCKOLDS E.R. — The activation of proaccelerin. **Brit. J. Haemat.**, 1965, **11**, 395.

[4] BLOMBÄCK B. — Purification and stabilization of factor V. **Nature**, 1963, **198**, 886.

[5] ESNOUF M.P., JOBIN F. — The isolation of factor V from bovine plasma. **Biochem. J.**, 1967, **102**, 660.

[6] HJORT P. — Intermediate reactions in the coagulation of blood with tissue thromboplastin. **Scand. J. Clin. Lab. Invest.**, 1957, **9**, suppl. 27, 183.

[7] JOBIN F., ESNOUF M.P. — Studies on the formation of the prothrombin-converting complex. **Biochem. J.**, 1967, **102**, 666.

[8] KAHN M.J.P., HEMKER H.C. — Studies on blood coagulation factor V. I : The interaction of salts of fatty acids and coagulation factors. **Thromb. Diath. Haemorrh.,** 1970 (in press).

[9] PAPAHADJOPOULOS D., HOUGIE C., HANAHAN D.J. — Purification and properties of bovine factor V ; a change of molecular size during blood coagulation. **Biochem.,** 1964, **3,** 264.

[10] PAPAHADJOPOULOS D., HOUGIE C., YIN E.T. — Purification and properties of bovine factor X ; molecular changes during activation. **Biochem.,** 1964 a, **3,** 1931.

[11] QUICK A.J. — On the constitution of prothrombin. **Amer. J. Physiol.,** 1943, **140,** 212.

[12] QUICK A.J. — On the quantitative relationship between calcium and prothrombin. **Amer. J. Physiol.,** 1947, **148,** 211.

[13] SURGENOR D.M., WILSON N.A., HENRY A.S. — Factor V from human plasma. **Thromb. Diath. Haemorrh.,** 1961, **5,** 1.

[14] WARE A.G., MURPHY R.C., SEEGERS W.H. — The function of Ac-globulin in blood clotting. **Science,** 1947, **106,** 618.

[15] WARE A.G., SEEGERS W.H. — Plasma accelerator factor and purified prothrombin activation. **Science,** 1947, **106,** 41.

[16] WEISS H.J. — A study of the cation- and pH-dependent stability of factors V and VIII in plasma. **Thromb. Diath. Haemorrh.,** 1965, **14,** 32.

## SUMMARY

A method is described to obtain a preparation of factor V from citrated human plasma. A purification of 30 times is achieved at a yield of 15 %. The contamination by other coagulation factors is minimal.

## RESUME

Une méthode est décrite pour obtenir une préparation de Facteur V à partir de plasma humain citraté. Une purification de 30 fois est réalisée avec un rendement de 15 %. La contamination par d'autres facteurs de coagulation est minime.

## ZUSAMMENFASSUNG

Es wird eine Methode zur Präparation von Faktor V aus menschlichem Plasma beschrieben. Es wird ein Gehalt von 15 % bei einer 30-fachen Reinigung erhalten. Die Verunreinigung durch andere Gerinnungsfaktoren ist minimal.

## RESUMEN

Se describe un método para obtener un preparado de Factor V a partir de plasma humano citratado. Se obtiene una purificación de treinta veces, con un rendimiento del 15 por ciento. La contaminación por otros factores de coagulación es mínima.

Резюме Описывается метод получения фактора V из цитратной плазмы человека. В результате 30-разовой очистки получили 15%. Загрязнение другими факторами коагуляции минимальное.

**FACULTE MIXTE DE MEDECINE ET DE PHARMACIE DE LYON**

**MAITRISE EN BIOLOGIE HUMAINE**

***Certificat d'études supérieures de génétique humaine générale***

Ce certificat est obligatoire pour l'obtention de la maîtrise en génétique. Il représente un certificat à option pour les maîtrises de cancérologie expérimentale, d'hématologie et d'immunologie.

L'enseignement théorique sera donné **chaque mercredi** de 9 heures à 12 heures et de 14 heures à 17 heures, du mercredi 7 janvier 1970 au mercredi 24 juin 1970, à la Salle Pasteur, Hôtel-Dieu.

1° Cours de 9 heures à 11 heures et de 15 heures à 17 heures.

2° Confrontations bio-cliniques de 11 heures à 12 heures.

3° Mathématiques et Statistiques appliquées à la génétique humaine générale de 14 heures à 15 heures.

L'enseignement pratique comprendra des stages d'immunogénétique, d'enzymologie génétique, de cytogénétique et d'informatique appliquée à la génétique par période bloquée et sur rendez-vous pour chaque élève. Ces stages débuteront **dès la première semaine du mois de novembre 1969.**

Des auditeurs libres sont admis à l'enseignement théorique, mais non aux stages pratiques.

Les demandes de pré-inscriptions et les demandes de renseignements seront reçues jusqu'au 1er novembre 1969 au secrétariat du service de génétique (Professeur agrégé J.-M. ROBERT), Hôtel-Dieu, Lyon-2e.

Les candidats retenus s'inscriront ensuite au secrétariat de la Faculté de Médecine, 8, avenue Rockefeller, 69 - Lyon-8e.